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ABSTRACT

The kinetics of decomposition of the pharmaceutical ranitidine (a major precursor of NDMA) during chloramination was investigated and some decomposition by-products were identified by using high performance liquid chromatography coupled with mass spectrometry (HPLC-MS). The reaction between monochloramine and ranitidine followed second order kinetics and was acid-catalyzed. Decomposition of ranitidine formed different by-products depending on the applied monochloramine concentration.

Most identified products were chlorinated and hydroxylated analogues of ranitidine. In excess of monochloramine, nucleophilic substitution between ranitidine and monochloramine led to by-products that are critical intermediates involved in the formation of NDMA, e.g. a carbocation formed from the decomposition of the methylfuran moiety of ranitidine. A complete mechanism is proposed to explain the high formation yield of NDMA from chloramination of ranitidine. These results are of great importance to understand the formation of NDMA by chloramination of tertiary amines.

KEYWORDS

NDMA, Nitrosamine, Chloramination, Disinfection By-products, Ranitidine

Introduction

Nitrosamines, especially N-nitrosodimethylamine (NDMA), form during disinfection of drinking waters at near nanogram per liter levels[1] or wastewaters at concentrations up to several hundred ng/L.[2] They are considered as probable human carcinogens by the US Environmental Protection Agency,[3] and are listed in the USEPA's Contaminant Candidate List 3.[4] They can be formed in the presence of nitrites and free chlorine (HOCl-enhanced nitrosation) but are preferentially formed during chloramines disinfection.[5] Over the last decade, several formation mechanisms have been proposed to explain NDMA formation by chloramination of secondary and tertiary amines. Most of them involve a nucleophilic substitution between dimethylamine (DMA) and monochloramine (NH_2Cl) to form an Unsymmetrical Dimethylhydrazine intermediate (UDMH).[6,7] Dichloramine ($NHCl_2$) was proposed to favor the production of NDMA, through the formation of a chlorinated UDMH (UDMH-Cl) intermediate instead of UDMH.[8] In the presence of bromide ion, it has been suggested that a brominated UDMH (UDMH-Br) would probably be formed.[9,10] These intermediates were never detected during experiments because they are expected to be rapidly oxidized to NDMA.

The contribution of tertiary amines to the production of substantial amounts of NDMA during chloramination has been pointed out.[2,11,12] In particular, the pharmaceutical ranitidine has been demonstrated to produce high yields of NDMA (> 60%).[11-13] Ranitidine is a histamine H2-receptor

antagonist used in treatment of peptic ulcer diseases, and was one of the most prescribed drug in the 80s. It has been progressively superseded by proton pump inhibitors, but it still remains in the top 15 sold-list of prescribed drugs in different European countries.[14] Ranitidine has been detected in European and US wastewaters at concentrations ranging from 220 ng/L to 550 ng/L.[15,16] Such high concentrations in wastewaters could explain the important NDMA formation potentials of wastewaters as compared to model waters containing similar amounts of DMA,[2] because of the higher conversion rate of ranitidine in NDMA (> 60% as compared to < 3% for DMA).[11,13] The presence of a pool of tertiary and quaternary amines acting as NDMA precursors (e.g., pesticides, pharmaceuticals and personal care products) could also participate in the overall NDMA yields observed in wastewaters.[13,17] Ranitidine has been identified in surface waters of Italy at concentrations ranging from 1 to 10 ng/L,[18,19] and has been detected in 1.2% of US streams at 0.01 µg/L.[20] Several studies have addressed the photochemical degradation of ranitidine in the environment.[21,22] Several photodecomposition products of ranitidine have been identified, but the by-products formed during the reaction between ranitidine and common oxidants used in water treatment (e.g., chlorine, monochloramine or ozone) have not been investigated.

Many kinetic studies have addressed chlorine reactivity with model compounds but the reactivity of monochloramine with simple model compounds is not well documented.[23] NDMA formation kinetics of some tertiary amines (i.e., ranitidine, chlorphenamine and doxylamine) have been recently investigated in various matrices.[24] In this study, real water matrices had a significant impact on NDMA formation kinetics, especially leading to an initial lag period because of competitive reactions between natural organic matter (NOM) and tertiary amines. Studies about the decomposition kinetics of NDMA precursors such as anthropogenic tertiary amines are lacking. Moreover, potential intermediate species involved in the formation of NDMA remain unidentified.

The aim of this study was to investigate the kinetics of decomposition of ranitidine by chloramination and to identify its decomposition by-products by using high performance liquid chromatography coupled with mass spectrometry (HPLC-MS). Reactions were conducted in deionized water to determine the kinetic constants for the reaction between monochloramine and ranitidine in pure solutions; hence

potential competitive effects of NOM with ranitidine were not studied. The identification of the reaction by-products should be useful to determine nucleophilic or electrophilic substitution sites in order to better understand the reaction mechanisms leading to the formation of NDMA by chloramination of ranitidine.

Materials and Methods

Materials. All experiments were conducted using deionized water (Milli-Q, Millipore) buffered with sodium acetate (pH = 4.0-5.5), a mixture of sodium phosphate monobasic and sodium phosphate dibasic (pH = 7.0-8.5), or sodium carbonate (pH = 10). pH values were adjusted as needed using sodium hydroxide or sulfuric acid (0.1 N, Fisher Scientific). Ranitidine was supplied through Sigma-Aldrich and was used without further purification. All other reagents were reagent grade or described previously.[13] All glassware used was washed with deionized water and baked at 500 °C for at least 5 hours prior to use.

Experimental Methods. Preparation of monochloramine stock solutions was previously described,[13] and is summarized in the Supporting Information (SI) Text S1. The concentration of monochloramine stock solutions was chosen to get the desired concentration in the working solution. Ranitidine solutions were prepared by dissolving a pre-determined amount of ranitidine in 1 L of 10 mM acetate, phosphate or carbonate buffer. 100 mL of monochloramine stock solution was then added to the working solution and reactions were conducted in amber glass bottles at 20 °C, under dark conditions to avoid the photolysis of NDMA. At given contact times, residual oxidants were quenched using a slight excess of sodium thiosulfate (2 g/L) and samples were transferred to glass vials for HPLC-MS^n analyses. Most of the experiments were performed using high initial concentrations of ranitidine in order to ensure full detection of the parent compound and its by-products. This level of concentrations was not representative of what can be detected in wastewater treatment plants or environmental samples.

Total Chlorine and Chloramines Analyses. Free chlorine and total chlorine concentrations in the sodium hypochlorite stock solutions were determined by iodometric titration with sodium thiosulfate 0.1

M (Prolabo, >99.9%). NH_2Cl and $NHCl_2$ concentrations were determined by spectrophotometric measurement using their respective molar extinction coefficients at 245 nm and 295 nm and solving simultaneous equations.[25] Residual oxidant was analyzed by iodometric titration.

Analyses of Ranitidine and its Chloramination By-products. High performance liquid chromatography coupled with diode array detection and mass spectrometry (HPLC-DAD-MS^n) analyses were performed with a Thermo Surveyor chromatographic system including two detectors: a Thermo Surveyor diode array detector and a Thermo DECA XP Plus ion trap mass spectrometer. Ranitidine and its chloramination by-products were separated using a Phenomenex Luna PFP2 column (250 × 4.6 mm, pore size: 100 Å, particle size: 5 µm). The mobile phase consisted in (A) formic acid/methanol (1:1000 v/v) and (B) formic acid/milliQ water (1:1000 v/v) pumped at a flow rate of 0.6 mL/min. Elution started at 5% of A for 5 min, increased to 30% of A in 20 min and holding for 5 min, then increased to 90% of A in 10 min and holding for 2 min, and then returned to initial conditions. Total run time was 60 min (including the conditioning of the column to the initial conditions). Injection volume was 100 µL. All samples were analyzed in full scan mode and MS^2 simultaneously. Chemical ionization was performed in atmospheric pressure chemical ionization mode (APCI), in positive and negative mode. The parameters were: capillary temperature of 250 °C, vaporizer temperature 450 °C, gas flow 95 u.a., auxiliary gas flow 56 u.a., corona discharge at 5 µA with a voltage 4.5 kV and capillary voltage 14 V. Mass range detection was 50-500 uma (to detect the formation of dimers). MS^2 experiments were performed on protonated molecular ions in order to identify by-products. MS^2 experiments were performed as follows: collision energy of 35%, Q activation of 0.25 and activation time of 30 ms. Analyses were performed in both APCI positive and APCI negative mode, but chloramination by-products of ranitidine were only detected in positive mode. For the determination of ranitidine decomposition kinetics, a series of ranitidine solutions at different concentrations (ranging from 0.05 µM to 2 µM) was analyzed in APCI positive mode to obtain a calibration curve ($R^2 = 1.000$).

Results and Discussion

Ranitidine Decomposition Kinetics at Several pH. The reaction of NH_2Cl with ranitidine was assumed to follow second-order kinetics, first-order with respect to each reactant. The rate of ranitidine decomposition in the presence of a large excess of monochloramine ($[NH_2Cl]_0/[RAN]_0 > 100$ mol/mol) can be considered as pseudo first-order with respect to ranitidine (equation 1):

$$-\frac{d[RAN]}{dt} = k_{obs}[RAN] \qquad (1)$$

where $k_{obs} = k_{app}\,[NH_2Cl]_0$

Ranitidine decomposition rates were determined at different pH from the reaction of 1.5 µM ranitidine with 200 µM NH_2Cl, using HPLC-MS analyses. The linear plots obtained between $\ln([RAN]/[RAN]_0)$ and reaction time confirmed the pseudo first-order rate with respect to the concentration of ranitidine (Figure 1). At pH < 5.5, ranitidine was instantaneously decomposed and kinetics could not be studied. Ranitidine chloramination rate decreased when increasing pH, indicating an acid-catalyzed decomposition (Figure 1). Ranitidine was found to exhibit a maximum NDMA formation yield around pH 8 after 5 days of reaction.[13] However, ranitidine decomposition did not show a maximum at pH 8. This finding indicates that the higher formation of NDMA at this pH and long contact times is not directly related to the decomposition rate of molecular ranitidine. Moreover, previous work demonstrated that the formation of NDMA was very slow (maximum NDMA formation occurring after 24h of contact time[13]) as compared to the fast decomposition rate of ranitidine observed in this study. The value of k_{app} at pH 7 was 34.9 $M^{-1}.s^{-1}$, which is much lower than the kinetic constants obtained for the chloramination of DMA ($7.98.10^8$ $M^{-1}.s^{-1}$)[26] and resorcinol ($7.5.10^5$ $M^{-1}.s^{-1}$)[23]. Steric hindrance could be responsible for the slower reaction of NH_2Cl with the DMA group of ranitidine as compared to DMA. Furthermore, chlorine transfer between NH_2Cl and DMA is subjected to general acid catalysis.[26,27] In a similar manner, chlorine transfer to the DMA group of ranitidine (i.e., electrophilic substitution) could be favored at acidic pH, which would explain the higher decomposition rate observed (Figure 1). Moreover, NH_2Cl decomposes at acidic pH by disproportionation and hydrolysis and thus may create species (e.g., $NHCl_2$ or HOCl) that enhance the decomposition of ranitidine.[28]

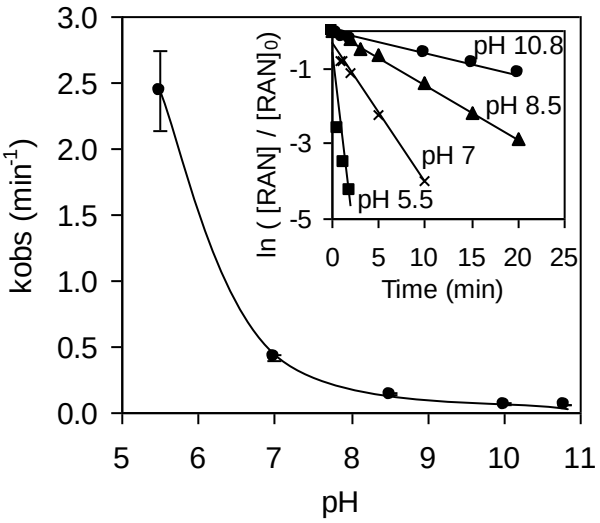
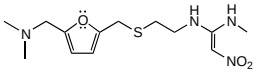
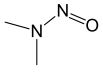
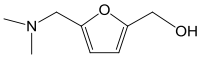
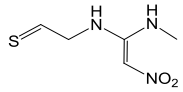
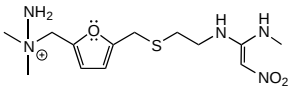
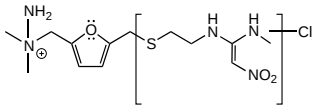
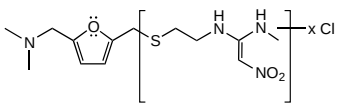
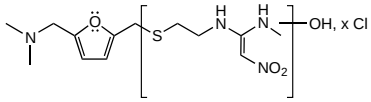
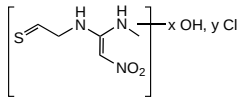
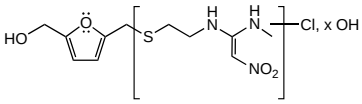


Figure 1. Ranitidine decomposition rates during chloramination at different pH. $[RAN]_0$ = 1.5 μM, $[NH_2Cl]_0$ = 200 μM.

Identification of Ranitidine By-products around Equimolar Conditions. Chromatographic and mass spectral data for ranitidine and its decomposition products analyzed by HPLC-MS are summarized in Table 1. Structures of decomposition products are proposed based on their MS and MS^2 spectral data. Attempts were made to identify the reaction by-products of 5-(dimethyl-aminomethyl)furfuryl alcohol (or DFUR, a molecular structure found in ranitidine and a major precursor of NDMA[10,11]) but they were probably too polar to be detected in our analytical conditions.

Table 1. Ranitidine reaction products detected by HPLC-MS in APCI positive mode. *By-products non-detected when reactions were stopped using sodium thiosulfate.

Compound	Fragmentation	Nominal mass	Structure	RT (min)
Ranitidine (RAN)	**315,** 270 (4 %), 176 (1.6%), 111 (2%)	314		24.1
NDMA	**75**	74		16.0
Dimethyl-aminomethyl furfuryl alcohol (DFUR)	**156,** 111 (38%)	155		8.0

thioethyl-N-methyl-2-nitroethene-1,1-diamine (P175)	**176,** 145 (10%), 130 (6%), 116 (4%)	175		16.6
RAN + NH_2 (P330)	**330,** 270 (5%), 113 (8%)	330		22.7
RAN + NH_2 + Cl (P364)	**364** (366), 304 (6%, 306), 223 (4%), 95 (4%)	364		22.1
RAN + Cl (P348)	**349** (351), 304 (3%), 223 (7%), 170 (3%)	348		24.8
RAN + 2 Cl (P382) *	**383** (385, 387), 349 (6%, 351), 170 (19%), 111 (10%)	382	**x=1 : m/z 349, x=2 : m/z 383**	38.8
RAN + OH	**331,** 286 (7%), 193 (29%), 154 (22%), 111 (34%)	330		12.7
RAN + OH + Cl	**365** (367), 227 (19%, 229), 181 (49%), 154 (32%), 111 (52%)	364	 **x=0 : m/z 331** **x=1 : m/z 365** **x=2 : m/z 399**	11.4
RAN + OH + 2 Cl *	**399** (401,403), 270, 215 (217), 167 (169), 154, 139, 111	398		40.0
P175 + OH (P191)	**192**	191		17.2
P175 + 2 OH + Cl (P241)	**242** (244), 192 (9%)	241	 **x=1, y=0 : m/z 192** **x=2, y=1 : m/z 242** **x=2, y=3 : m/z 310**	39.6
P175 + 2 OH + 3 Cl	**310** (312, 314), 192 (9%), 181 (13%), 145 (8%)	309		24.4
RAN - DMA + 2 OH + Cl (P337)	**338** (340), 320 (12%), 259 (37%), 227 (31%), 192 (41%), 181 (86%), 111 (154%)	337		23.9
RAN - DMA + 3 OH + Cl (P353)	**354** (356), 259 (10%), 249 (53%), 185 (22%), 152 (14%), 111 (17%)	353	**x=1 : m/z 338, x=2 : m/z 354**	26.5

Ranitidine and monochloramine were introduced in the reaction buffer at 167 µM and 400 µM, respectively (similar concentration range), to identify the first compounds produced by ranitidine decomposition in the presence of low concentrations of NH_2Cl. Major ions produced were chlorinated and/or hydroxylated derivatives, i.e., m/z 331 (hydroxylated ranitidine), m/z 349 (chlorinated ranitidine), m/z 365 (chlorinated and hydroxylated ranitidine), m/z 383 (ranitidine with two chlorine atoms) and m/z 399 (hydroxylated ranitidine with two chlorine atoms) (see SI, Figure S1). These products can result

168 from chlorination and further oxidation of the N-methyl-2-nitroethene-1,1-diamine group. As proposed
169 by Joo and Mitch for the chloramination of monomethylamine, chlorine attack on nitrogen atom and
170 oxidation leads to the formation of organic chloramines and hydroxylamines.[29] The presence of m/z 399
171 can be attributed to the subsequent chlorine substitution on N-methyl or ethene double bond or on the
172 sulfur atom. Some other by-products were detected in smaller amounts. Experiments were carried out
173 without quenching residual oxidant at the desired reaction time in order to investigate the potential
174 influence of sodium thiosulfate on the by-products stability, because sodium thiosulfate can break N-Cl
175 bonds formed after chlorination.[30] Only products containing two chlorine atoms (i.e., molecular ions m/z
176 383 and m/z 399) were not detected when sodium thiosulfate was added. All the other by-products were
177 detected with and without sodium thiosulfate addition. MS^2 experiments were conducted on ranitidine
178 and the above-mentioned by-products to determine the position of chlorine substitution and
179 hydroxylation. Figure S2a in SI gives the MS^2 spectrum obtained for ranitidine. A loss of dimethylamine
180 (DMA) group (45 Da) gave the fragment ion m/z 270, and the following loss of NO_2 radical ion (46 Da)
181 generated the radical fragment ion m/z 224. Different ruptures of C-S bonds led to the formation of
182 fragments m/z 176, 144 and 124. These results are in accordance with MS^2 fragments observed in a
183 previous study by Radjenović et al.,[31] using a quadrupole-time of flight (Q-Tof) detection. In the same
184 study, the compound with molecular ion m/z 331 has been identified as a photocatalytic by-product of
185 ranitidine.[31] The difference of 16 Da was attributed to the hydroxylation of ranitidine. MS^2 experiments
186 on this molecular ion revealed similar fragments than those observed by Radjenović et al.[31] (See SI,
187 Figure S3b). The typical loss of DMA group led to the fragment m/z 286 and further loss of water led to
188 the fragment m/z 268, which confirms the hydroxylation of ranitidine. Different ruptures of C-S bonds
189 in the molecular ion m/z 331 generated pairs of fragments m/z 156 and 176, and fragments m/z 188 and
190 143. Fragment ion m/z 156 can be attributed to the previously mentioned DFUR, i.e. the hydroxylated
191 dimethylaminomethylfuran group. Dehydroxylation of DFUR led to the fragment m/z 138 (Figure S3).
192 By comparing the MS^2 fragments of chlorinated ranitidine (m/z 349) with that of ranitidine (m/z 315),
193 several similarities could be observed (see SI, Figure S2). Losses of DMA and NO_2 groups from

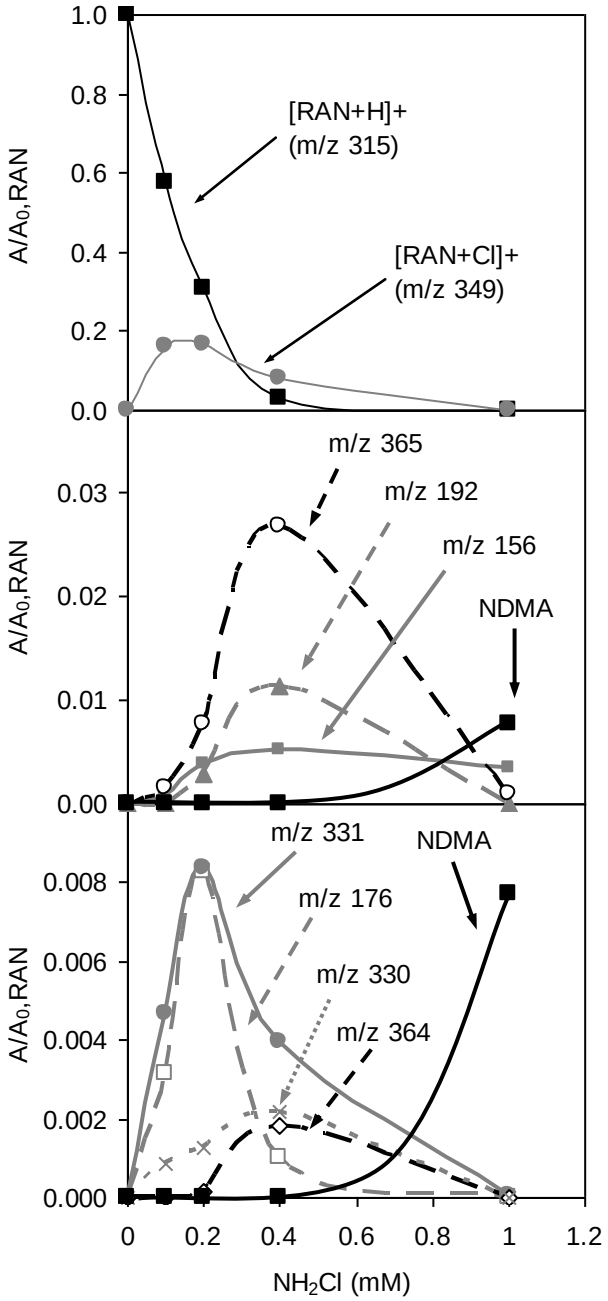
chlorinated ranitidine generated chlorinated fragments m/z 304 and 258. This indicates that chlorine transfer did not occur on the dimethylamino group as it was previously suggested during chloramination of tertiary amines.[32] This implies that chlorine transfer leading to the release of DMA is not a pathway of NDMA formation by chloramination of ranitidine. Moreover, the major fragment ion of chlorinated ranitidine was m/z 210. This fragment is the chlorinated analogue of the fragment ion m/z 176 of ranitidine, which indicates that chlorine substitution does not occur on the furan group but probably on nitrogen or sulfur atoms. This is confirmed by the fact that a chlorinated analogue of m/z 124 (i.e. the dimethylaminomethylfuran fragment) was not detected.

Some minor by-products exhibited a gain of 15 Da as compared to molecular ranitidine and chlorinated ranitidine. Chromatograms exhibited small peaks with a molecular ion m/z 330 (intermediate product P330) and a molecular ion m/z 364 (P364) at retention times of 22.7 min and 22.1 min, respectively. P364 was identified as the chlorinated analogue of P330. The observation of such products is consistent with the occurrence of a nucleophilic substitution between NH_2 group of monochloramine and the DMA group of ranitidine, leading to a gain of 15 Da as compared to ranitidine (i.e. P330) (Scheme 1). Moreover, the odd nominal mass of this product indicates an odd number of nitrogen atoms, confirming the gain of a nitrogen atom as compared to ranitidine. The relatively low abundance of this peak suggests that it is rapidly decomposed to other degradation by-products. MS^2 experiment conducted on P330 generated the same fragments as ranitidine (i.e., m/z 270, 258, 224, 176 and 124, Figure S3). This indicates that the fragmentation of P330 leads to the loss of the NH_2 group, probably because of a weak bond. MS^2 fragmentation of the chlorinated analogue of P330 did not provide any additional information.

Influence of NH_2Cl Concentration. The reaction between ranitidine (167 μM) and various concentrations of NH_2Cl (ranging from 0 to 1 mM) after 2h of reaction time at pH 8 (with 10 mM phosphate buffer) was monitored using HPLC-MS (Figure 2). Decomposition rate of ranitidine increased with increasing NH_2Cl concentration until full degradation for concentrations greater than 0.5

220 mM. The formation of chlorinated ranitidine (i.e., m/z 349) decreased with increasing NH_2Cl
221 concentration. Maximum chlorinated ranitidine formation occurred when ranitidine and NH_2Cl were
222 introduced in equimolar concentrations.

223 Most of the major by-products were preferentially formed for a NH_2Cl/ranitidine ratio of
224 approximately 2 mol/mol (e.g., m/z 176, 192, 330, 331, 364, 365) (Figure 2). By-products with
225 molecular ions m/z 176 (P175) and m/z 192 (P191) were detected at retention times of 16.6 min and
226 17.2 min respectively. P191 was identified as the hydroxylated analogue of P175 (i.e. the thioethyl-N-
227 methyl-2-nitroethene-1,1-diamine moiety). NDMA formation occurred only for a NH_2Cl concentration
228 of 1 mM, i.e. in a large excess of NH_2Cl, and after disappearance of the other by-products. Products
229 P330 and P364 (postulated as resulting from nucleophilic substitution on the DMA group of ranitidine)
230 were totally degraded at NH_2Cl concentrations where NDMA formed (i.e., 1 mM), observation
231 consistent with their potential implication as intermediate compounds involved in the formation of
232 NDMA.



233

234 **Figure 2.** Influence of NH_2Cl concentration on the decomposition of ranitidine and the formation of
235 ranitidine by-products. $[RAN]_0$ = 167 µM, t = 2 h, pH = 8. Relative scale based on initial ranitidine
236 concentration.

237 **Ranitidine By-products Formed in Excess of Monochloramine.** In order to determine the by-
238 products formed in the presence of an excess of monochloramine, i.e. in the conditions where NDMA
239 formation is favored, the decomposition of ranitidine (12 µM) was investigated in the presence of 2.5
240 mM NH_2Cl over 48 h at pH 8 (with 10 mM phosphate buffer). These conditions have been

demonstrated to maximize the formation of NDMA.[13] Decomposition of ranitidine was complete in less than 2 min, while the formation of NDMA was much slower (Figure 3a). Chlorine transfer (i.e., the formation of chlorinated ranitidine) was very fast and chlorinated ranitidine was entirely decomposed in less than 2 min as well as ranitidine. NDMA formation reached a plateau after 75 min. This is in agreement with our previous observations of NDMA formation in similar conditions and monitored by GC/MS.[13] Products that were previously detected when NH_2Cl was introduced at equimolar concentrations or with a slight excess as compared to ranitidine (e.g., fragments m/z 156, 330, 331, 364, 365, Figure 2) were not detected, probably because they were rapidly decomposed in the presence of a large excess of NH_2Cl (Figure 3b).

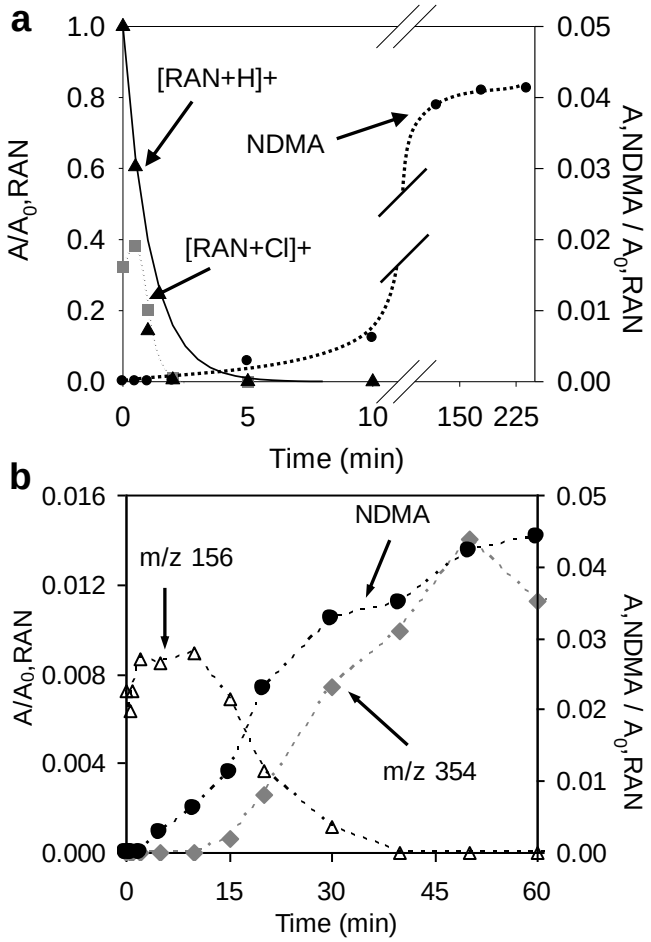


Figure 3. Decomposition of ranitidine (a) and formation of NDMA and other by-products (a and b) by chloramination monitored by HPLC-MS in APCI positive mode. $[RAN]_0 = 12$ µM; $[NH_2Cl]_0 = 2.5$ mM;

253 pH = 8 with 10 mM phosphate buffer. Relative area is based on initial ranitidine concentration. Solid
254 line (a) represents model values of ranitidine decomposition based on the rate constant obtained at pH 8.

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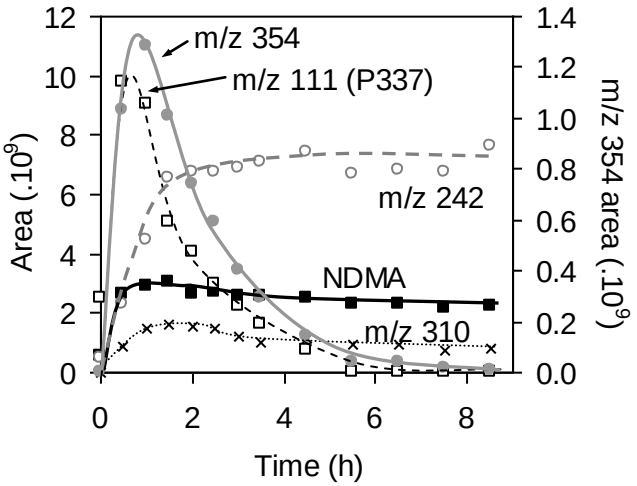


256

257 **Figure 4.** Formation of NDMA and other by-products by chloramination of ranitidine monitored by
258 HPLC-MS in APCI positive mode. $[RAN]_0$ = 120 µM; $[NH_2Cl]_0$ = 10 mM; pH = 8. Lines represent best
259 fits of data.

260 Figure 4 depicts the formation of ranitidine by-products for higher initial concentrations (i.e., 120 µM
261 of ranitidine and 10 mM of NH_2Cl) and longer contact times. Different products were slowly formed
262 along with NDMA (m/z 111, 242, 310, and 354). NDMA (m/z = 75) and products with molecular ions
263 m/z 242 and m/z 310 reached a plateau after 30 min of contact time. The product with a molecular ion
264 m/z 242 (P241) was generated by hydroxylation and chlorination of P175, probably on nitrogen atoms
265 of the N-methyl-2-nitroethene-1,1-diamine moiety as previously mentioned for molecular ranitidine.
266 Subsequent chlorination of P241 led to the product with a molecular ion m/z 310 (the presence of 3
267 chlorine atoms was confirmed by its isotopic distribution).

268 The ion m/z 111 was identified as the major fragment of the molecular ion m/z 338 (P337) (see Table
269 1). The odd nominal mass of this product indicates an odd number of nitrogen atoms, reflecting the loss
270 of the DMA group. MS spectra of this product revealed the presence of a chlorine atom and a gain of 32
271 Da. This molecule can be attributed to the dihydroxylation and chlorination of ranitidine after the loss of

the DMA group (corresponding to fragment m/z 270). The product with molecular ion m/z 354 (P353) was identified as a hydroxylated analogue of P337, thus explaining the short delay between the formations of these products (Figure 4). P337 and P353 reached a maximum after around 1 h of reaction and then slowly decreased. Their formation was strongly correlated to NDMA formation during the first 45 min of reaction. Hence, these compounds may be products resulting from carbocation intermediates formed during the last step of NDMA formation, as discussed below.

Influence of Free Chlorine. The influence of free chlorine (180 μM HOCl) on ranitidine (180 μM) was investigated to compare chlorination and chloramination by-products produced at pH 8 and after 2 h of contact time. Similar chlorinated and hydroxylated by-products (i.e., molecular ions m/z 156, 176, 192, 331, 349, 365) were formed after chlorination and chloramination and exhibited similar responses. However, P330 and P364 (chlorinated analogue of P330) were not detected in the presence of HOCl. This confirms the hypothesis of a nucleophilic substitution between NH_2Cl and the DMA group of ranitidine, leading to the P330 intermediate. Subsequent electrophilic substitution of P330 gives the chlorinated analogue P364.

Proposed NDMA Formation Pathway

During chloramination of amines, either chlorine transfer or nucleophilic substitution can occur. Chlorine transfer from NH_2Cl to the nitrogen atom of the DMA group of ranitidine is unlikely to occur as a predominant pathway because it would only lead to the formation of DMA or dimethylchloramine (DMCA) that are minor precursors of NDMA (i.e., < 3% molar yields).[2,6,7] Several tertiary amines have been demonstrated to produce important yields of NDMA, especially ranitidine (> 60% molar yield),[11-13] and more recently dimethylbenzylamine (64% molar yield).[17] Hence, a chlorine transfer (i.e., electrophilic substitution) cannot explain the high yields of NDMA obtained for those tertiary amines.

The formation of NDMA by chloramination of DMA was previously proposed to occur via the formation of an UDMH, UDMH-Cl or UDMH-Br intermediate, followed by an oxidation in the

298 presence of dissolved oxygen.[6,7,9,10] This last step of the mechanism remains quite unclear because the
299 kinetics of UDMH oxidation have not been clearly investigated in the presence of both dissolved oxygen
300 and NH_2Cl. UDMH (m/z 61) or equivalent intermediates UDMH-Cl (m/z 95) and UDMH-Br (m/z 139)
301 were not detected in our analysis conditions. They were probably not separated correctly by liquid
302 chromatography because of their low molecular weight. Moreover, they are expected to be rapidly
303 oxidized to NDMA in the presence of dissolved oxygen and monochloramine or free chlorine, which
304 explains why they have never been observed when NDMA was formed during chlorination or
305 chloramination of water solutions containing amines.[7,8] Experiments were conducted to investigate the
306 formation of NDMA by oxidation of UDMH (500 nM) by NH_2Cl (2.5 mM) in the presence of dissolved
307 oxygen. Molar yields after 24h of contact time were very low (i.e., < 0.01 %) as compared to NDMA
308 formation from ranitidine (i.e., > 60%). Our results are in accordance with several studies that
309 investigated UDMH oxidation by dissolved oxygen or NH_2Cl.[33-35] Hence, these results suggest that
310 UDMH is not likely to be a major intermediate involved in the formation of NDMA during
311 chloramination, especially from tertiary amines such as ranitidine.

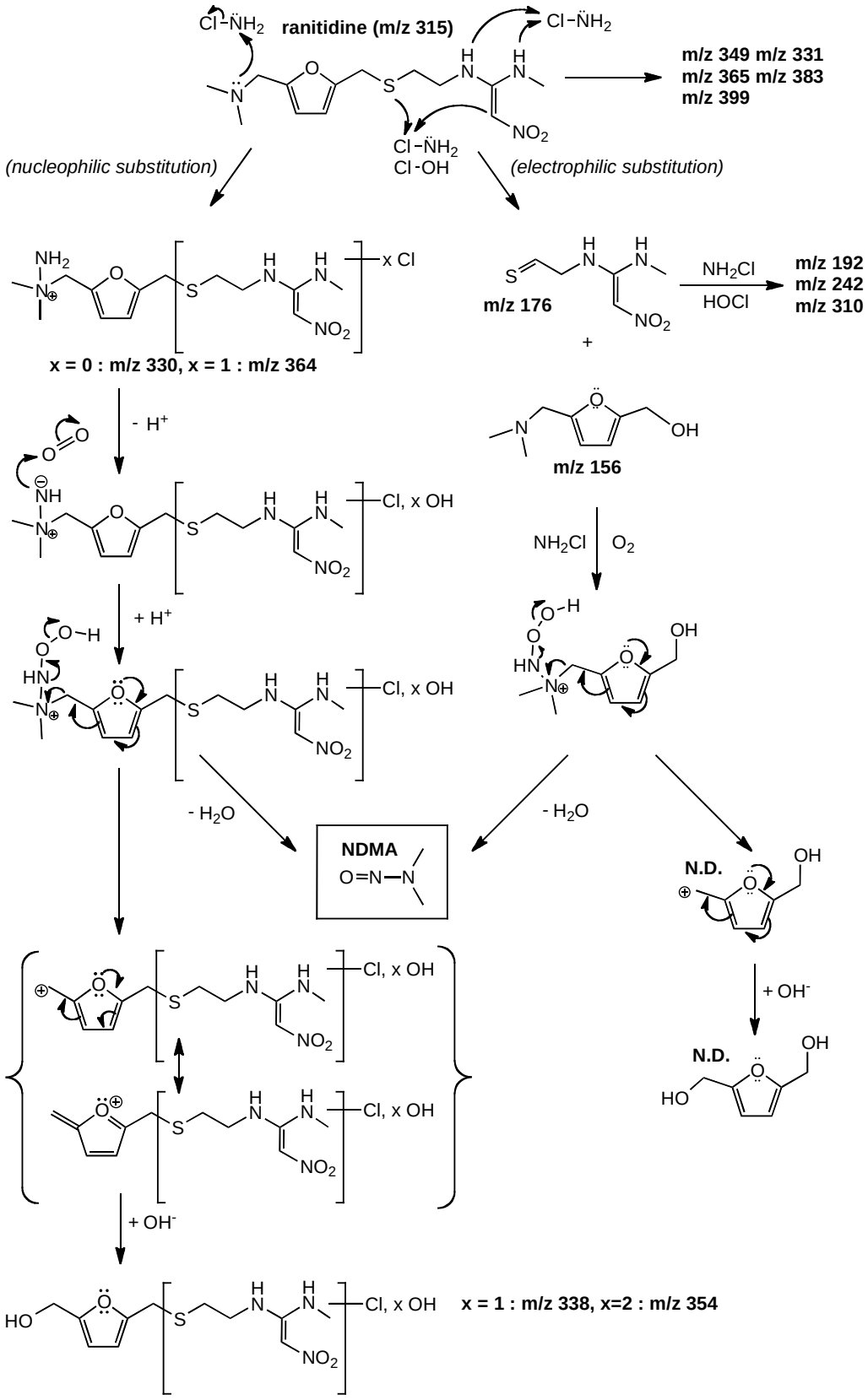
312 Based on these observations, we propose that DMA groups must be attached at the benzylic position
313 of aromatic or heterocyclic rings in order to produce high yields of NDMA. Indeed, as shown in **Scheme**
314 **1**, the release of NDMA from ranitidine leads to the formation of a stable carbocation at benzylic
315 position of the furan ring that is favored thermodynamically. These carbocation intermediates are prone
316 to react with nucleophiles such as water and thus may lead to the observed products P337 and P353 after
317 hydroxylation on the methylene group. This mechanism is in accordance with the simultaneous
318 production of P337, P353 and NDMA observed during chloramination of ranitidine (Figure 4).

319 In a previous study, we demonstrated that almost no NDMA was formed in the absence of dissolved
320 oxygen during chloramination of ranitidine.[13] Because the formation of UDMH and its oxidation by
321 dissolved oxygen is not likely to occur, dissolved oxygen incorporation has to occur directly on the
322 intermediate formed after the reaction between NH_2Cl and the DMA group of ranitidine (i.e. products
323 P330 or P364). Hence, we propose that the positive charge on the nitrogen atom of the DMA moiety

reduces the pKa of hydrogen on the NH_2 group, therefore favoring the formation of a highly reactive NH^- intermediate which reacts with dissolved oxygen to yield a NDMA precursor group.

We hypothesize that nucleophilic substitution rather than chlorine transfer is the main reaction occurring on the DMA moiety of ranitidine. In this case the steric hindrance brought by the two methyl groups probably disfavors the transfer of the bulky chlorine atom of NH_2Cl to the amine. However, chlorine transfer is likely to take place on less hindered moieties of ranitidine, especially on nitrogen atoms of the thioethyl-N-methyl-2-nitroethene-1,1-diamine moiety, producing the chlorinated analogues of ranitidine (i.e., m/z 349 and m/z 383) and then hydroxylated analogues after further oxidation. Chlorine transfer can also take place on the sulfur atom and cause sulfoxide compounds formation,[36] or the cleavage of the C-S bond leading to the release of the observed product P175 and 5-(dimethylaminomethyl)furfuryl alcohol (DFUR, m/z 156) (see SI, Scheme S1). Our results are consistent with three initial reactions: i) fast chlorine transfer leading to chlorinated analogues of ranitidine, ii) the cleavage of the C-S bond leading to DFUR and iii) nucleophilic substitution leading to P330. The proposed pathways are also probably interconnected because chlorinated ranitidine (P348) can also react with NH_2Cl through nucleophilic substitution and lead to P364, and both hydroxylated and chlorinated ranitidine analogues can liberate DFUR through the cleavage of C-S bond. DFUR is known to be a decomposition product of ranitidine,[37] and to produce important amounts of NDMA as well as ranitidine (i.e., > 50% molar yields).[10,11] Hence, DFUR produced via chlorine addition on ranitidine and C-S bond cleavage could react with NH_2Cl to contribute to the overall formation of NDMA. The stable carbocation (i.e. methylfurfuryl alcohol) that would form along with NDMA via this pathway and its hydroxylated analogue were not detected, probably because they were not properly separated in our chromatographic conditions.

Scheme 1. NDMA formation mechanism proposed for the chloramination of ranitidine (N.D. = Not Detected).



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351

352 **Implications for Water Treatment**

353 The kinetics study revealed that ranitidine decomposition was favored at acidic pH, while NDMA
354 formation reaches a maximum around pH 8.[13] Hence, NDMA formation cannot be directly related to the
355 decomposition of molecular ranitidine. The influence of pH on NDMA formation depends on complex
356 reactions involving monochloramine stability, the potential formation of chloramines decomposition
357 products (e.g., peroxynitrite ions or hydrazine intermediates), or acid dissociation constants of ranitidine
358 and its by-products. Even if the disproportionation of NH_2Cl to $NHCl_2$ has been proposed to favor the
359 formation of NDMA from the reaction with DMA,[8] the decomposition of NH_2Cl at acidic pH is
360 expected to limit the production of NDMA in the case of ranitidine oxidation.[13] Moreover, no analogue
361 to P330 with a chlorine atom on the amine group (i.e., RAN + NHCl) was detected, as it could be
362 expected to form from the reaction of ranitidine with dichloramine. In our experimental conditions (i.e.,
363 pH 8), the production of $NHCl_2$ was limited. Hence, $NHCl_2$ does not seem to play a major role in the
364 formation of NDMA during chloramination of ranitidine, as we already proposed in a previous study.[13]
365 Chlorine transfer between NH_2Cl and DMA is also subjected to general acid catalysis.[25,27] In a similar
366 manner, chlorine transfer to the DMA group of ranitidine (i.e., electrophilic substitution) could be
367 favored at acidic pH, which would explain the higher decomposition rate observed (Figure 1), and thus
368 would limit the occurrence of a nucleophilic substitution and subsequent NDMA formation. Hence, the
369 formation of NDMA from ranitidine during water treatment processes could be reduced by favoring
370 electrophilic substitution (i.e., chlorine attack) at pH < 7.
371 Many by-products were identified during the chloramination of ranitidine. Different compounds are
372 produced depending on NH_2Cl:ranitidine ratio and reaction time. Nucleophilic substitution of DMA
373 group is not affected by C-S bond cleavage and formation of chlorinated and hydroxylated analogues of
374 ranitidine. Thus NDMA formation occurs through multiple pathways, which explains the high yields
375 observed. In real water disinfection conditions, ranitidine (or another major precursor of NDMA) is
376 expected to be at very low concentrations (i.e. at ng/L levels) as compared to NH_2Cl concentrations.
377 Hence, NDMA formation from ranitidine is likely to be maximized in these conditions and could only

be limited by lowering the pH or by reducing the initial concentration of ranitidine (or other NDMA precursors).

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Supporting Information. Additional details of the materials and methods, additional figures (chromatogram and MS spectra of ranitidine and several by-products) and scheme of P155 (DFUR) and P175 formation.

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SYNOPSIS TOC art.